AN ELECTRON MICROSCOPIC STUDY OF THE STRUCTURE AND FORMATION OF RED PIGMENT GRANULES IN HAIR FOLLICLES*

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Hair color is determined to a large extent by the quantity and type of pigment present in the keratinized cells of the hair shaft. The pigment of the keratinized cells is derived from melanocytes, the most important function of which is the synthesis and transfer of pigment granules into the differentiating hair cells.

Several authors have studied with the electron microscope the production of black pigment granules (melanin) in the melanocytes of hair follicles (1, 2, 3, 4). The formation of black pigment granules is a complex process: Small vesicles, premelanosomes (3, 5, 6), containing parallel arrays of inner membranes, are found first in the Golgi region of the melanocytes. The vesicles grow in size and a melanin polymer accumulates on the inner membranes. This structure is designated as the melanosome. As melanization proceeds, the membranes of the vesicles are masked and the final product, the melanin granule, appears as a structureless, uniformly dense body.

Red hair pigment has received scant attention in the past and relatively little is known about its mode of formation, structure and chemical nature. Birbeck and Barnicot have made a few observations on pigment-forming cells of human red hair follicles (1, 2). They noted vesicles in the Golgi region possessing distinct, often concentric shells of dense material similar to those seen during initial stages of black pigment granule formation. The mature granules seemed to be composed of loosely aggregated small particles; these appeared round, or slightly ovoid and in general smaller than the mature black pigment granules.

The purpose of the present study has been to obtain information on the structure and mode of formation of red pigment granules of the hair follicle. In order to recognize differences in the fine structure and mode of formation of red and black pigment granules, an electron microscopic investigation of pigment-forming cells of the hair follicle of both black and red haired guinea pigs was undertaken. In this paper new information is given on the structure and production of red pigment granules. Furthermore, while certain findings of previous workers are confirmed, a few new observations are also presented on developmental forms of black pigment granules.

MATERIALS AND METHODS

Male guinea pigs, weighing 300–500 gm. with pure black or red fur were obtained from Rockland Farms, New Jersey. The hair follicles were dissected singly and intact from the skin and were immersed in ice cold 1% or 2% osmium tetroxide buffered to pH 7.4–7.6 with veronal acetate buffer (7). After fixation for two hours, the tissues were dehydrated in ethanol and embedded in epoxy resins according to the method of Luft (8). Sections were cut with a Porter-Blum microtome, stained with lead hydroxide (9) and examined in RCA EMU 3F electron microscope.

RESULTS

One of the most characteristic properties of black pigment-producing cells of the hair follicle is the presence of a well-developed Golgi complex and numerous melanin granules in various stages of formation. The Golgi complex of melanocytes of the guinea pig hair follicle consists of many parallel arrays of agranular membranes and smooth surfaced vesicles. Premelanosomes, melanosomes and mature melanin granules are seen in the Golgi region as well as in other parts of the cytoplasm (Fig. 1, 2). Premelanosomes appear round in cross sections and rod shaped in longitudinal sections. These consisted of a smooth-walled vesicle containing many parallel oriented inner membranes (Fig. 1, 2). The melanosomes are somewhat larger than premelanosomes and have a roundish or oval appearance depending on the direction of sectioning. Their most characteristic property is the presence of many dense and rela-

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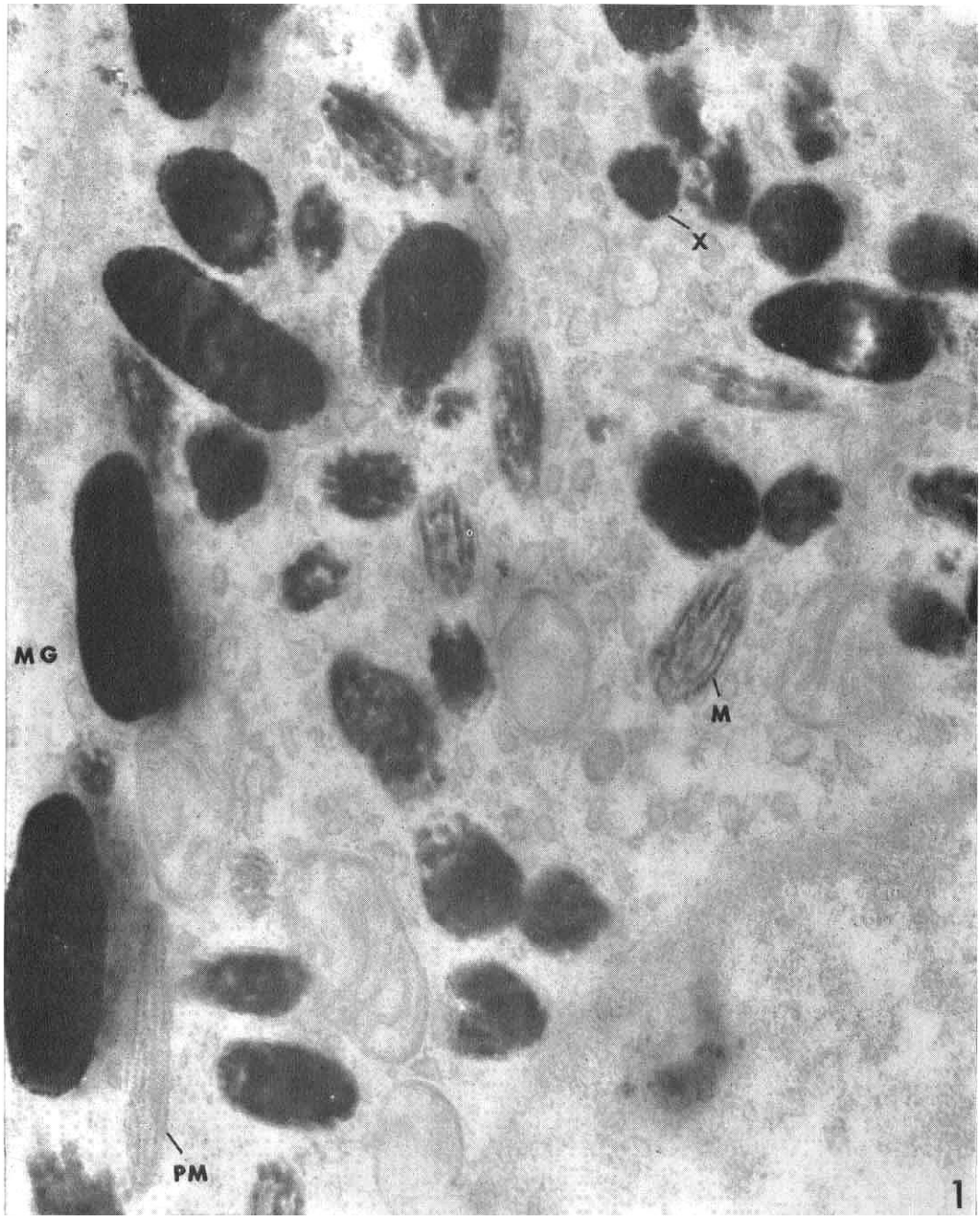

FIG. 1. Electron micrograph of a portion of a black melanocyte showing different stages in the formation of melanin granules. PM: Premelanosome showing oriented parallel array of membranes. M: Melanosome revealing thick membranes with the accumulation of pigment. MG: A mature melanin granule appearing uniformly dense. X: A granule which represents an intermediate stage between the melanosome and a mature melanin granule. The body of the granule is divided into several compartments by electron dense partitions. × 45,080.

FIG. 2. Electron micrograph of a part of a black melanocyte. Melanosomes and mature melanin granules are distributed in the cytoplasm. MG: Mature melanin granule. M: Melanosome. Y: Shows an anomalous melanin granule. While one part of the granule shows no internal structure and resembles a mature melanin granule, the other part shows only partial melanization and appears similar to a melanosome. × 72,520.

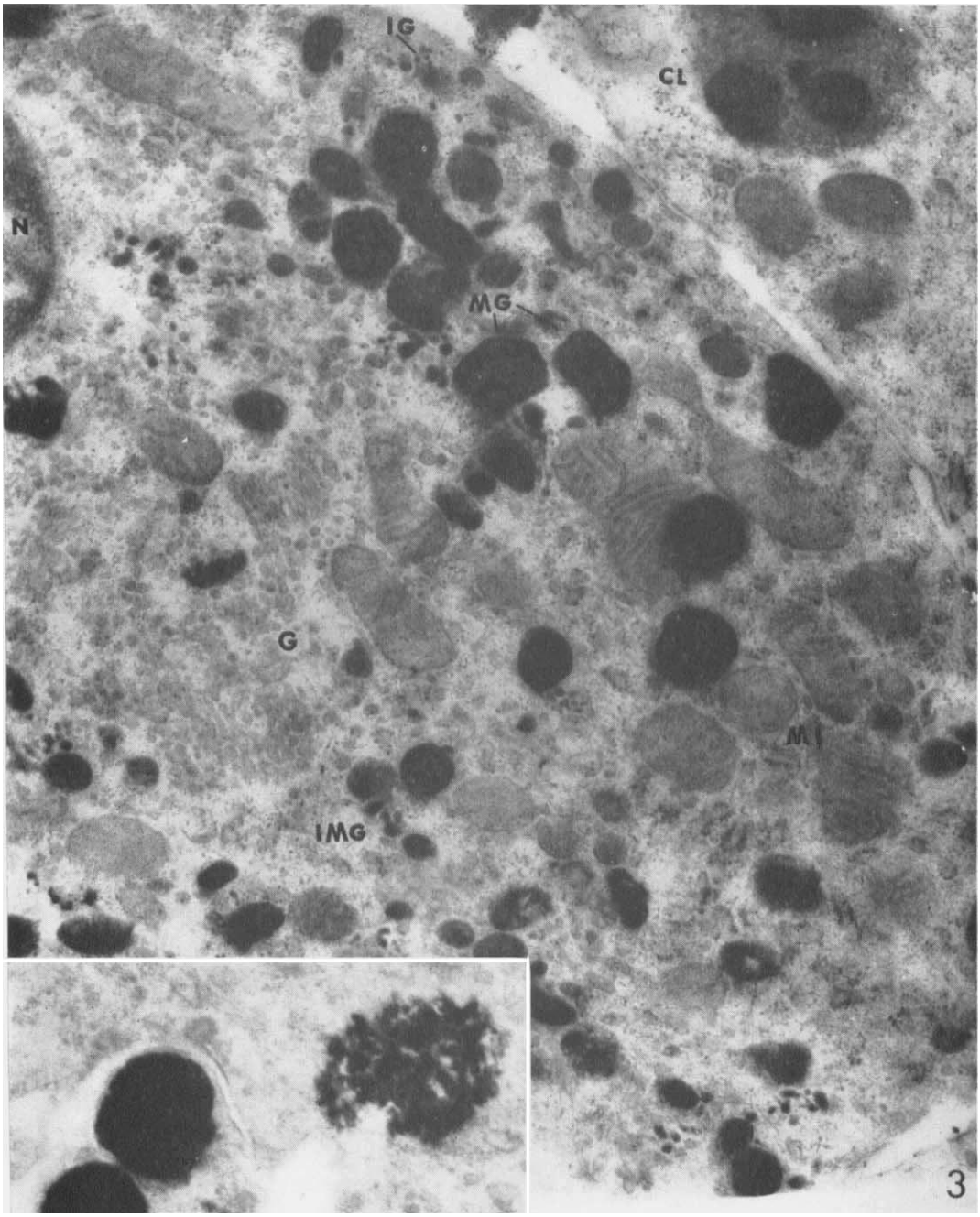

FIG. 3. The larger part of the electron micrograph shows part of a red melanocyte. In the upper right corner a portion of a keratinizing cell is seen. A portion of the nucleus of the red melanocyte (N), a well-developed Golgi complex (G) and many mitochondria (M) are shown. IG: A small granule representing the earliest recognizable stage in the formation of red pigment. These granules are about 400 Å in diameter. IMG: Larger granules showing intermediary stages of red pigment formation. MG: Mature red pigment granules. CL: A clump of three red pigment granules are shown in a neighboring keratinizing cell. Inset: One granular and two homogeneous appearing red pigment granules. × 28,224.

tively thick inner membranes containing the newly deposited melanin polymer (Fig. 1, 2). The mature granules are rod-shaped and appear as dense structureless bodies enclosed by a thin limiting membrane (Fig. 1, 2).

In addition to the forms described above, melanin granules of different structural characteristics are also noted. One is a roundish melanin granule divided into several compartments by electron-dense partitions (Fig. 1). Another kind of granule resembles in one part the structure of a mature melanin granule while in the other part that of a melanosome (Fig. 2).

Both the mode of formation and the structure of red pigment granules appear quite different from that of black pigment granules. While there is a well-developed Golgi complex in the red pigment-producing cells, premelanosomes are not seen, and the red pigment granules seem to be formed without an oriented precursor structure. Red pigment granules appear first as small, discrete particles measuring approximately 400 Å in diameter. These uniformly dense, round bodies are occasionally encased by a thin limiting membrane. Such granules occur not only in the Golgi region but throughout the cytoplasm (Fig. 3). In addition, larger granular and homogeneous granules are numerous in the Golgi region and at scattered points of the cytoplasm (Fig. 3). The larger granules are considered to represent intermediary and mature developmental forms of red pigment. The mature red pigment granules occur singly or in clumps of two or three. Furthermore, aggregates of red pigment granules are seen in the cytoplasm of presumptive cortical and medullary cells.

DISCUSSION

The results of the present study confirm previous findings on the structure and mode of formation of black pigment granules in the melanocytes of hair follicles (1, 2, 3). The roundish melanized granule, noted to be divided into several compartments, is a new form that has not been previously described. We think that this type represents an intermediary stage between the melanosome and the mature melanin granule. The other type structurally resembled both a melanosome and a mature granule. This type of structure suggests that melanization in certain cases may proceed at different rates in different parts of the developing granule.

Red pigment granules, as noted in this study, appear to be not only structurally different from black pigment granules, but basic differences also seem to prevail with regard to their mode of formation. Structurally, red pigment granules are either granular or homogeneous appearing roundish structures whereas mature black pigment granules are dense structureless rod-shaped bodies. Concerning the mode of formation, the most striking difference appears to be that the production of red pigment granules proceeds without formation of an oriented skeleton. Whether there is a complete lack of a skeleton, or if it occurs in a disorganized form not recognizable by electron microscopy, cannot be resolved at the present time. In regard to this problem, Moyer (10, 11) has noticed that the inner membranes of melanosomes of the mouse retinal pigment epithelium undergo a variety of structural changes in accordance with genetic factors. When the mutant allele is present in the homozygous condition at the P locus, the inner membranes of melanosomes become highly disorganized. In view of this, it is probable that some allelic change in the pigment controlling genetic loci might produce a disorganized supporting framework in the red pigment-forming cells of the hair follicle of the guinea pig. Perhaps further experimentation will clarify this problem and will lead to a better understanding of the mode of formation of red pigment granules.

SUMMARY

1. The fine structure and mode of formation of black and red pigment granules in the hair follicles of guinea pigs were studied with the electron microscope.

2. Developmental forms of black pigment, the premelanosome, melanosome and melanin granule are described. In addition to these, two new forms are described for the first time.

3. Red pigment granules are produced without the formation of a precursor structure, such as the premelanosome. The initial granule is a small, round particle, measuring about 400 Å in diameter. Intermediate and mature granules appear round and have a granular or homogeneous structure.

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DISCUSSION

Dr. Alvin S. Zelickson (Minneapolis, Minn.): I wish to thank Dr. Parakkal for his interesting presentation. We too have been studying red pigment granules, but in human hair rather than in guinea pig hair follicles. Our findings differ from those presented this morning. We find a definite membrane structure during the formation of the granules, similar to that described in the early stages of black pigment formation.

Dr. Paul F. Parakkal (in closing): Drs. Birbeck and Barnicot have pointed out (1, 2) that the formation of red pigment is similar to that of the black pigment. However, we find that in guinea pigs the red pigment formation follows a different pattern from that of the black pigment. It is interesting to note that morphologically human red pigment is different from that of the red pigment formed in the guinea pig.